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Local Treatment of Abdominal Wound Reduces Tumor Implantation

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Background and Objectives: Pneumoperitoneum increases the trocar-site tumor implantation rate using a human colon cancer cell line in a hamster model. The purpose of this study was to determine whether local treatment of trocar sites with potential tumoricidal agents can inhibit tumor implantation after pneumoperitoneum.

Methods: GW-39 human colon cancer cells (0.5 ml of 2.5% v/v; 8.0×10^5 cells) were injected throughout the abdomen of 133 Golden Syrian hamsters through a midline incision. The animals were randomized to receive either untreated 5-mm trocars in each abdominal quadrant (group I control, n = 49), trocars dipped in 10% povidone-iodine (group II, n = 53), or trocars coated with 1% silver sulfadiazine (group III, n = 51). The midline wounds were also coated with the respective agents before closing. Pneumoperitoneum was then maintained at 10 mmHg for 10 min, after which the trocar wounds were closed. In group II, the trocar sites were treated with a coat of povidone-iodine after the trocars were withdrawn and before closing. Gross and microscopic tumor implants were analyzed at 7 weeks postoperatively.

Results: The rate of tumor cell implantation at trocar sites was reduced from 93% (172/184) in the control group to 75% (126/168) and 78% (141/180) in groups II and III, respectively ($P < 0.0001$). Fewer palpable tumors were detected in groups II and III (40% and 23%, respectively) than in the control group (72%, $P < 0.0001$). Mean tumor mass in group III (0.4 ± 0.1 g), but not in group II (1.0 ± 0.2 g), was significantly less than that in the control group (1.3 ± 0.1 g, $P < 0.01$). Overall tumor involvement of the larger midline wound was similar for all groups (I = 80%, II = 79%, III = 71%). However, palpable tumors were identified more frequently in group I (67%) than in groups II and III (43%, $P < 0.05$; 22%, $P < 0.01$, respectively).

Conclusion: Pretreatment of abdominal wounds with povidone-iodine or silver sulfadiazine can reduce tumor implantation after pneumoperitoneum in a hamster model. *J. Surg. Oncol. 1998;69:9–14.*

KEY WORDS: **laparoscopy; colon cancer; implants; metastasis; trocar; ports**

INTRODUCTION

Laparoscopic colectomy for curable colon cancer is currently under investigation. Numerous case reports of tumor implants at trocar sites have been published [1]. The etiology of these abdominal wall implants is unclear and could be attributable to disseminated disease [2]. The

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precise incidence of trocar implantation is unknown; estimates of 4% exist [1]. This compares unfavorably with the 1% incidence of abdominal wall implants seen in open procedures [3]. A recent review of laparoscopic colectomy for cancer by the Clinical Outcomes of Surgical Therapy (COST) study group, who are performing the National Institutes of Health (NIH)-funded trial comparing open and laparoscopic colectomy, showed a rate of 1% [4]. Pneumoperitoneum used in laparoscopy has been shown to enhance the deposition of free, intraperitoneal viable tumor cells into trocar site wounds as local isolated recurrences in an animal model [5]. Previous studies demonstrated that 10 mmHg pneumoperitoneum significantly increased tumor implantation at trocar sites [5,6]. Even so, there is controversy over the role of pneumoperitoneum on the implantation of tumor at a trocar site. Aerosolization of cells by the pneumoperitoneum during laparoscopic procedures has not been confirmed. Local factors may influence the development of tumor at small abdominal wall wounds. Since there are few studies examining methods to inhibit tumor implantation at trocar sites, this study was performed to determine if local treatment of trocar sites with cytotoxic agents, namely povidone-iodine and silver sulfadiazine, could inhibit tumor implantation after pneumoperitoneum.

MATERIALS AND METHODS

Preparation of Colon Cancer Cell Line

Preparation of the GW-39 human colon cancer cell suspension was previously described [3]. In summary, GW-39 human colon carcinoma, maintained in the thigh musculature of immunocompetent male Golden Syrian hamsters, was harvested, minced, and washed with McCoy's 5A medium three times before centrifugation at 386*g* for 10 min. The tumor pellet was frozen at −196°C in liquid nitrogen at a concentration of 50% (v/v) in McCoy's 5A medium and 20% dimethylsulfoxide (DMSO.) At the time of use, the pellet was thawed, washed three times in McCoy's 5A medium, and diluted to a concentration of 2.5% (v/v). An aliquot of GW-39 tumor cells were stained with trypan blue vital dye, counted, and assessed for viability. Preinjection cell viability ranged from 90% to 95% for all experiments.

Animal Model

Golden Syrian hamsters aged 6–10 weeks were anesthetized with 2.5% Fluorothane® (Wyeth Ayerst Laboratories, Philadelphia, PA). The abdominal wall was shaved and prepared with povidone-iodine. A 1-cm midline vertical incision was made through the midabdomen. Four 5-mm trocars (Ethicon-Endosurgery, Cincinnati, OH) were inserted into the right upper, right lower, left upper, and left lower quadrant of the anterior abdominal wall under direct vision. Tumor cells suspended in McCoy's 5A medium (0.5 ml) were injected throughout the peritoneal cavity through the 1-cm midline incision. The human colon cancer GW-39 yielded an estimated 6.7×10^7 cells per gram. Approximately 8.0×10^5 tumor cells were injected into each hamster (0.5 ml of a 2.5% cell suspension). The midline incision was closed with a single layer of running absorbable suture. The abdomen was then insufflated with carbon dioxide (10 mmHg) through the right lower quadrant 5-mm port for 10 min before removing the trocars in sequence from right upper, right lower, left lower, to left upper quadrant. The trocar wounds were closed with a single layer of figure-8 4-0 Vicryl™ sutures (Ethicon, Somerville, NJ).

A total of 153 animals were randomized to receive either untreated 5-mm trocars–one in each abdominal quadrant (group I control), trocars coated with 10% povidone-iodine (group II), or trocars coated with 1% silver sulfadiazine (group III). The midline incision was also coated with the respective agents before closing. In group II, the trocar abdominal wound sites were also treated with a coat of povidone-iodine after the trocars were withdrawn but before closing the wound. The studies were conducted in accordance with the guidelines established by the Animal Care Committee at Washington University under an approved animal studies protocol.

Postoperative Protocol

The animals were maintained on a hamster chow and water diet throughout the experiment. After 7 weeks, animals were sacrificed, weighed, and examined for evidence of tumor implantation. A 1-cm^2 section of the abdominal wall surrounding the trocar sites in the right upper, right lower, left upper, left lower quadrants, and midline incision was examined and excised. Gross tumor in the abdominal wall wound sites or in the intra-abdominal organs was harvested and weighed. Tissue samples without obvious tumor were subjected to histologic analyses using frozen, serially sectioned samples. Hematoxylin and eosin (H&E) staining was performed [7]. Tissue samples microscopically scored as questionable or not containing tumor by H&E were then stained with (1) Mayer's mucicarmine stain for mucin [7]; and (2) immunoperoxidase using an avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector, Burlingame, CA) and monoclonal antibody (MAb) 45-9, an anti-carcinoembryonic antigen (CEA) MAb [8]. Since GW-39 colon carcinoma cells secrete mucin and express CEA antigen while normal muscle express neither, these approaches helped to specifically detect small amounts of tumor implanted in the wound sites. The sections were reviewed for presence or absence of tumor by three observers, all blinded to the treatment groups.

Data Analysis

The null hypothesis for this study states that tumor implantation of 2.5% suspension of GW-39 colon cancer

TABLE I. Local Treatment of Abdominal Trocar Wounds: Hamster Groups and Weights*

	Group I Control	Group II Povidone-iodine	Group III Silver sulfadiazine
No. of hamsters	49	53	51
No. of deaths	3	11	6
No. of hamsters evaluated	46	42	45
Preoperative weight (g)[a]	145 ± 3	148 ± 4	142 ± 5
Postoperative weight (g)[a]	168 ± 4	170 ± 3	176 ± 5

*All P = not significant.
[a]Mean ±SEM.

is unaffected by the local application of povidone-iodine or silver sulfadiazine to abdominal wounds. In order to achieve a power of 90, b of 0.10, a of 0.05, and 95% confidence interval (95% CI) at a minimum difference of 20% regarding the effect of pneumoperitoneum, 50 hamsters, each with four independent trocar sites, were required for each group. The InStat Biostatistics software program (GraphPad Software, San Diego, CA) was used for data analyses. The frequency of tumor implantation at trocar sites and at midline laparotomy sites was compared between groups by two-tailed Fisher's exact test. Comparison of the mass (grams) of gross tumor implants at the wound sites was performed using the two-tailed Student's *t*-test. Statistically significant differences were defined as $P < 0.05$. All data are presented as mean ± standard error of mean (±SEM).

RESULTS

The number of randomized hamsters and deaths in each group is listed in Table I. There were more deaths in the treated groups. The higher number of deaths in the povidone-iodine group occurred early in the experiment when a larger volume of povidone-iodine was used to treat the wounds before closure. The resulting chemical burn caused an ileus, distention, and death within 3 days in seven animals. The amount of antiseptic was reduced and the toxicity was eliminated. A similar phenomenon was noted in the silver sulfadiazine-treated group when a larger volume of cream was used to coat the wounds early in the experiment.

The rate of tumor cell implantation at trocar sites was significantly reduced from 93% (172/184) in the control group to 75% (126/168) and 78% (141/180) in groups II (povidone-iodine) and III (silver sulfadiazine), respectively ($P < 0.0001$; Fig. 1). Fewer palpable tumors were detected in groups II (67/168, 40%) and III (42/180, 23%) than in the control group (133/184, 72%,$P < 0.0001$; Fig. 2). Mean trocar site tumor mass in group III, but not in group II, was significantly less than that of the control group ($P < 0.01$; Fig. 3). Overall tumor involvement of the larger midline laparotomy wound was similar for all groups; however, palpable tumors were identified less frequently in groups II and III than in group I (Fig. 4). The mean midline tumor masses were similar among the three groups: group I = 0.9 ± 0.2 g, group II = 0.8 ± 0.3 g, group III = 0.5 ± 0.1 g.

DISCUSSION

Since the introduction of laparoscopic colectomy for colon cancer, several case reports have documented metastasis to trocar sites within weeks to months after a potentially curable operation [1,9–12]. Often, metastatic deposits are to port sites other than the specimen extraction site, suggesting that factors other than direct seeding are responsible [13,14]. The present authors have previously shown that pneumoperitoneum contributes to the development of implants [5]. The goal of this study was to determine if tumor implantation at trocar sites (and midline laparotomy sites) can be inhibited by local treatment of trocar sites with cytotoxic agents after pneumoperitoneum.

GW-39 human colon carcinoma cells injected into the hamster peritoneum has proved a good model for study-

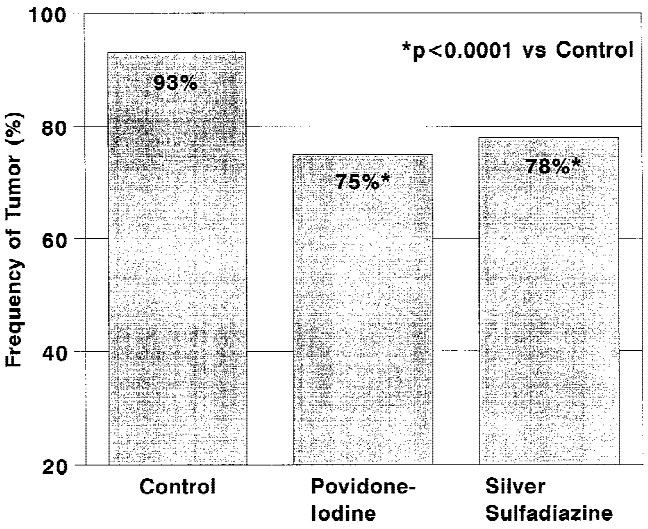

Fig. 1. Rate of overall trocar site tumor implantation: Control group, 172/184; Povidone-Iodine group, 126/168, Silver Sulfadiazine group, 141/180.

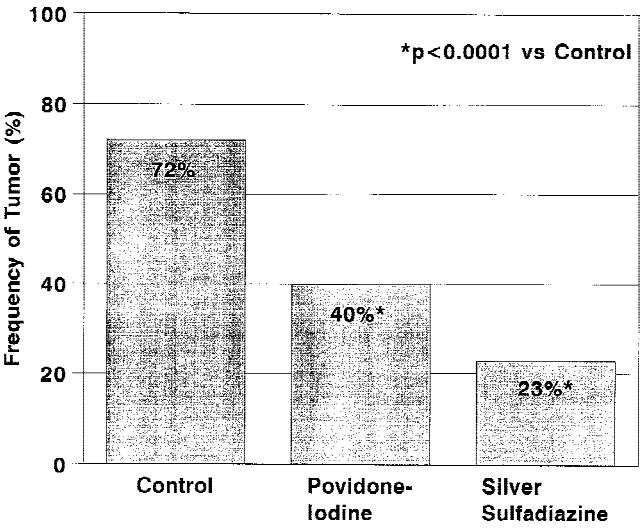

Fig. 2. Rate of palpable tumor implants at trocar sites: Control group, 133/184, 72%; Povidone-Iodine group, 67/168, 40%; Silver Sulfodiazine group, 42/180, 23%.

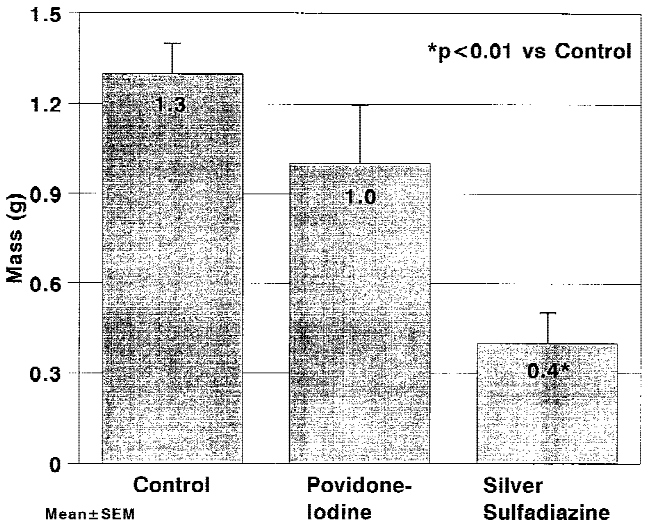

Fig. 3. Mass of palpable tumor implants at trocar sites.

ing the mechanical intraperitoneal spread of cancer [5]. Hematogenous metastases rarely occur in this tumor model, and intraperitoneal injection of disaggregated tumor cells simulates tumor spillage that may occur during dissection, manipulation, resection, and extraction of tumor during an operation in a human. In the current study, 2.5% tumor cell inoculum was used (~800,000 cells) to approach a concentration of tumor cells that may be found in the clinical setting; this proved an excellent tumor model for trocar site implantation [6].

Both in vitro and in vivo studies have shown povidone-iodine to be cytotoxic to human breast cancer cell and colorectal cancer cell lines [15–17]. Iodine released from povidone-iodine is an irreversible oxidant of essential cellular enzymes and causes rapid cytotoxicity [16]. Clinically, many surgeons (up to 70% of surgeons in Great Britain) have been irrigating intraluminally with povidone-iodine just before resection in hopes of preventing tumor recurrence of colorectal cancer [17]. Others employ perioperative wound lavage with povidone-iodine during colorectal or breast cancer surgery to reduce the local recurrence rate [15,18,19]. No studies, however, have examined the possibility for this agent to prevent tumor implants in abdominal trocar wounds after pneumoperitoneum. In addition to povidone-iodine, the topical antimicrobial silver sulfadiazine has been shown to have cytotoxic effects on lymphocytes, neutrophils, and fibroblasts [20,21]. Silver has been shown to possess tumoricidal properties against in vitro colon cancer cells SW1116 in experiments performed by one of the current authors during the 1970s (J.M. Connett, personal communication).

This study clearly showed that there was a significant reduction of overall and palpable trocar-site tumor implantation rate after coating the trocars with potential tumoricidal agents, namely povidone-iodine and silver sulfadiazine, before insertion of the trocar into the abdominal wall. The timing of the inhibition of tumor implants is important. In a previous study, tumor implantation was found to occur during pneumoperitoneum (probably due to mechanical spread) and after pneumoperitoneum (probably due to wound adherence) [22]. Thus, treating the potential tumor implant sites before pneumoperitoneum, as well as after pneumoperitoneum in the case of povidone-iodine, seems appropriate. Not only is povidone-iodine potentially cytotoxic, helping to reduce the implantation of tumor cells to the abdominal wall, but one could argue that the application of the povidone-iodine using a swab after pneumoperitoneum could cause a mechanical clearance of tumor cells from the abdominal wound. However, in the silver sulfadia-

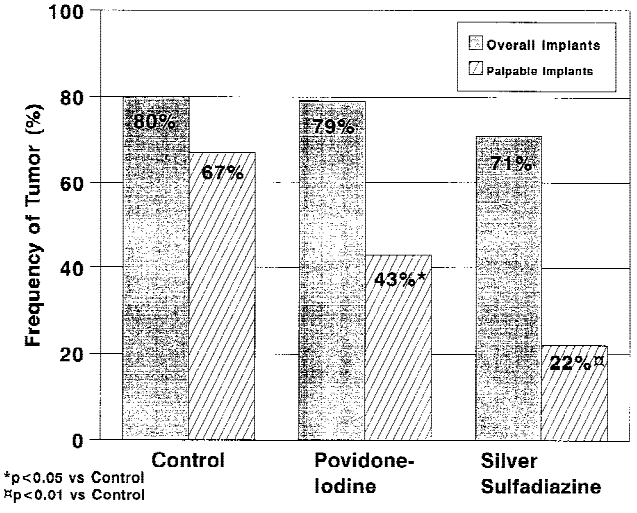

Fig. 4. Rate of midline tumor implantation.

zine-treated group, there was no second application of the material; thus, mechanical clearance of tumor cells definitely did not play a part in reducing the tumor implantation rate at the trocar wound sites using this particular agent. It is interesting that not only did silver sulfadiazine decrease the tumor implantation rate, but it also resulted in smaller palpable size tumors (one-third of the mass) compared with controls, further suggesting that it possesses chemopreventive properties.

There was less inhibition of tumor implantation at the midline laparotomy incision. This may be due to a decreased local concentration of these agents in the larger midline wound, and the presence of povidone-iodine at the time of incision because of the skin preparation. Further experiments are required to quantify the amount of these tumoricidal agents required to completely prevent tumor implantation, with or without pneumoperitoneum.

Only an approximately 20% reduction in tumor implantation was noted in the treated groups. Even though the agents applied may be considered tumoricidal, their action must be local in this model. Only enough povidone-iodine or silver sulfadiazine were applied to treat a 5-mm-diameter wound. The reminder of the abdomen is thus untreated. Since the agents act not only locally but temporarily during the postoperative period, viable cells within the abdomen may be deposited at the wound sites in subsequent days. This is also seen in a group of hamsters that underwent excision of their wounds [22]. The implantation of tumor cells at a trocar site cannot be completely eliminated by a temporary local treatment if viable cells remain accessible to the healing wound. Thus systemic or intraperitoneal treatment would be necessary to address this problem.

A higher concentration of povidone-iodine may have resulted in a lower implantation rate, but the accompanying toxicity seen with a larger amount applied may be prohibitive. The study did not address dosing issues. Should trocar site implantation become a problem in the clinical setting (and not prohibit the use of laparoscopic techniques on that basis), a dosing curve study would be required to define the optimum amount of agent to apply locally to a trocar site wound to prevent implantation of tumor.

CONCLUSION

A local application of existing, potentially tumoricidal, agents to trocar sites results in a reduction of tumor implantation in a hamster model using a human colon cancer cell line. This suggests that the use of well-tolerated solutions (povidone-iodine, silver sulfadiazine) may be useful as local chemopreventive agents for laparoscopy in colorectal cancer.

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COMMENTARY

The "Achilles heal" of laparoscopic management of abdominal tumor is the risk of implantation of cancer cells at trocar sites in combination with pneumoperitoneum. Anecdotal case reports have stimulated a more scientific approach to this problem; the results of this analytical direction are exemplified by the work of Wu and colleagues, as they describe the effects of the hamster model, using the human colon cancer cell GW-39. This reproducible model has been used to show the effect of manipulations on the trocar site, which, at least in the animal, reduce the microscopic adhesiveness of this particular cell line. The effect of pneumoperitoneum and trauma to the abdominal wall in these animals most likely plays a role in the enhancement of tumor cell implantation, although other mechanisms and local wound characteristics such as hematoma formation most likely play a role in the clinical setting. The ultimate protective mechanism is the immunologic competence of the patient, which may ultimately protect the abdominal wall from development of tumor growth. One of the major factors may be the technical skill of the surgeon, which, unfortunately, is the most difficult variable to measure in the uncontrolled environment of clinical cancer care.

The experimental design outlined by the group at Washington University will help develop new methods to quantitate the effect of manipulations performed during laparoscopic colon resection from malignancy. Unfortunately, animal models can only go so far in elucidating the mechanisms of the disease process. The ultimate answer, as the authors allude to, will be provided by well-done controlled trials of laparoscopic colon cancer resection. Until these answers are provided, the risk of trocar site recurrence mandates that patients be treated by experienced surgeons in the controlled environment of a clinical trial overviewed by the institutional review board.

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